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Wet STEM in SEM for Morphological Characterization of Novel Bacterial Species: *Vibrio galatheae* and *Photobacterium galatheae*

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Scanning electron microscope (SEM) is increasingly used for morphology characterization in various research fields. The technique is appealing due to the fact that it has a fairly large field of view (µm to mm) with nm lateral resolution, which makes it suitable for characterization of micrometer scale objects, which have nm scale features, such as bacteria. The major limiting factors for SEM as a visualization tool of bacteria are the sample preparation, which is prone to artefacts, and the sample interaction volume, which limits the lateral resolution. Here, we present an approach for morphology characterization, which involves minimal sample preparation and maximized lateral resolution: the use of environmental SEM (E-SEM) with a scanning transmission electron detector (STEM); wet STEM. We use wet STEM for morphological characterization (identification of cell shape, size and appendices) of two bacteria isolated from a mussel collected in the Solomon Sea (Solomon Islands) during the Danish research expedition Galathea 3: *Vibrio galatheae*[1] and *Photobacterium galatheae*[2].

The bacteria were grown in marine broth in flask cultures. After 24h growth at 25˚C the marine broth was washed with miliQ water and a 0.5 µL droplet placed on a plasma treated carbon film 200 mesh copper grid. The sample was negatively stained with 2 % uranyl acetate aqueous solution and imaged in an FEI Quanta 200 FEG E-SEM with an electron beam with spot 3 accelerated to 15 keV. During electron microscopy the bacterial cells were kept fully hydrated by using water as auxiliary gas and continuously condensing water on the sample. The bacteria were imaged using the transmitted electrons and a STEM detector (a 2 quad, diode back scattered electron detector mounted below the sample).

The wet STEM micrographs revealed that the *Vibrio galatheae*, has Vibrio monotrichous cells, 1.482±0.365 µm long, 0.743±0.181 µm width and 3.625±0.639 µm long flagellum (Figure 1), and the *Photobacterium galatheae* has Bacillus monotrichous cells , 2.261±0.404 µm long, 0.985±0.128 µm wide and 4.306±1.094 µm long flagellum (Figure 2). Hence, we show that the wet STEM in SEM analysis is a quick and efficient technique that can be easily used for identification of cell shape, appendices and size measurements of bacteria.

References

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2. *Photobacterium galatheae* sp. nov., a bioactive bacterium isolated from a mussel in the Solomon Sea, Henrique Machado, Sonia Giubergia, Ramona Valentina Mateiu and Lone Gram, International Journal of Systematic and Evolutionary Microbiology (2015), 65, 4503–4507.

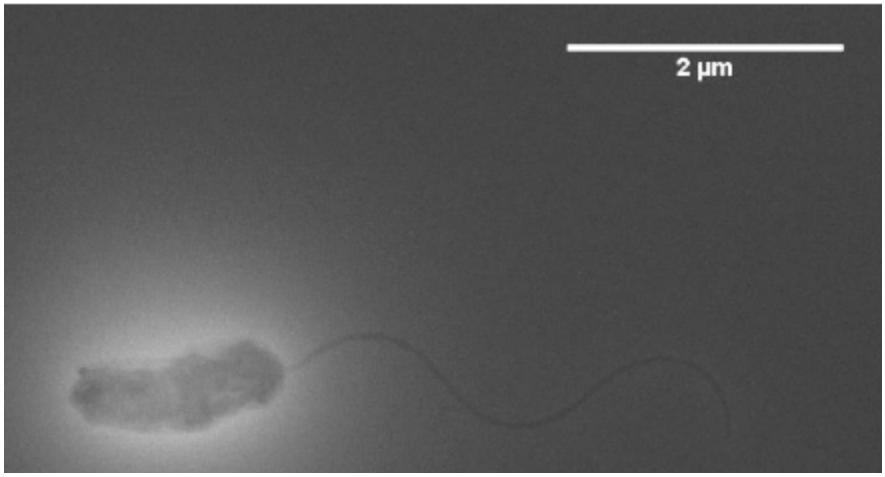

Figure 1. Wet STEM micrograph of a *Vibrio galatheae* cell.

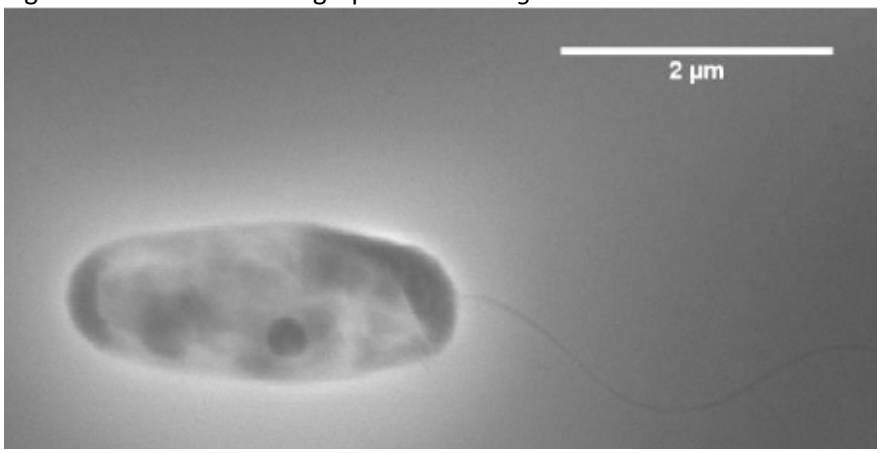

Figure 2. Wet STEM micrograph of a *Photobacterium galatheae* cell.